✡ DNA Polymerase:

- Principle enzyme of DNA replication
- In prokaryotes ⇒ 5 different DNA pol.
- In eukaryotes ⇒ 13 different DNA pol.

→ Prokaryotic DNA pol.

Activities	DNA pol I (cell activities) [300–400 copies]	DNA pol II (cell activities) [30–40 copies]	DNA pol III (DNA replication) [approx. 10 copies]
• 5'→3' polymerization	Yes	Yes	Yes
• 3'→5' exonuclease	Yes	Yes	Yes
• 5'→3' exonuclease	Yes	No	No
• Structure	Single polypeptide chain	Single polypeptide chain	Multimeric complex
• Discoverer	Arthur Kornberg (1956) (Noble prize)	Thomas Kornberg (1970) (father → son)	Thomas Kornberg & Malcolm Gefter

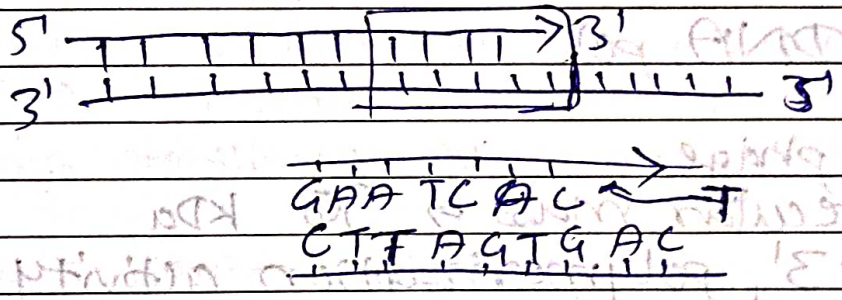
Eukaryotic DNA pol.

- α
- β
- γ
- δ
- ε

} Most essential

- θ
- ζ
- κ
- λ
- μ
- Rev-I
- η

→ Mode of action.



DNA pol I

- Isolated from E.coli
- More than 300-400 copies in an organism
- Molecular mass is 109 kDa.
- single pp chain
- 923 amino acids

1 — 323 — 324 — 923

small fragment (5'→3' exonuclease) | larger fragment (5'→3' polymerization, 3'→5' exonuclease)

Klenow fragment 68 kd

→ Application
- & cDNA formation.
- Random primer method
- Nick translation
- Used in Sangers method
- End Billing
- End labelling

✱ T4 DNA Pol.

- T4 phage.
- Single polypeptide chain
- Molecular mass = 104 KDa.
- 5'→3' polymerization activity
- 3'→5' exonuclease activity

✱ T7 DNA pol.

- T7 phage
- Molecular mass ⇒ 96 KDa
- 5'→3' polymerization activity
- 3'→5' exonuclease activity.

→ Sequenase
- T7 DNA pol but can't perform 3'→5' exonuclease activity.
- High processing capacity.
- Sequences DNA strand.

✱ Thermostable DNA pol.
→ Taq. polymerase
- from Thermophilus aquaticus
- 5'→3' polymerization activity
- 95 KDa
- No proof reading of DNA strand

→ Pfu DNA pol.

- Pyrococcus furiosus
- Magnesium ions
- 5'→3' polymerization activity
- 3'→5' exonuclease activity

→ Pfu (exonuclease−) DNA pol.

- 5'→3' polymerization activity
- no 3'→5' exonuclease activity
- High processivity

→ Vent DNA pol

- Thermococcus litoralis (isolated from)
- Mg^{+2} ions
- 5'→3' polymerization activity
- 3'→5' exonuclease activity
- Temp. stability is high compared to Taq pol.

→ Vent (exo−) DNA pol

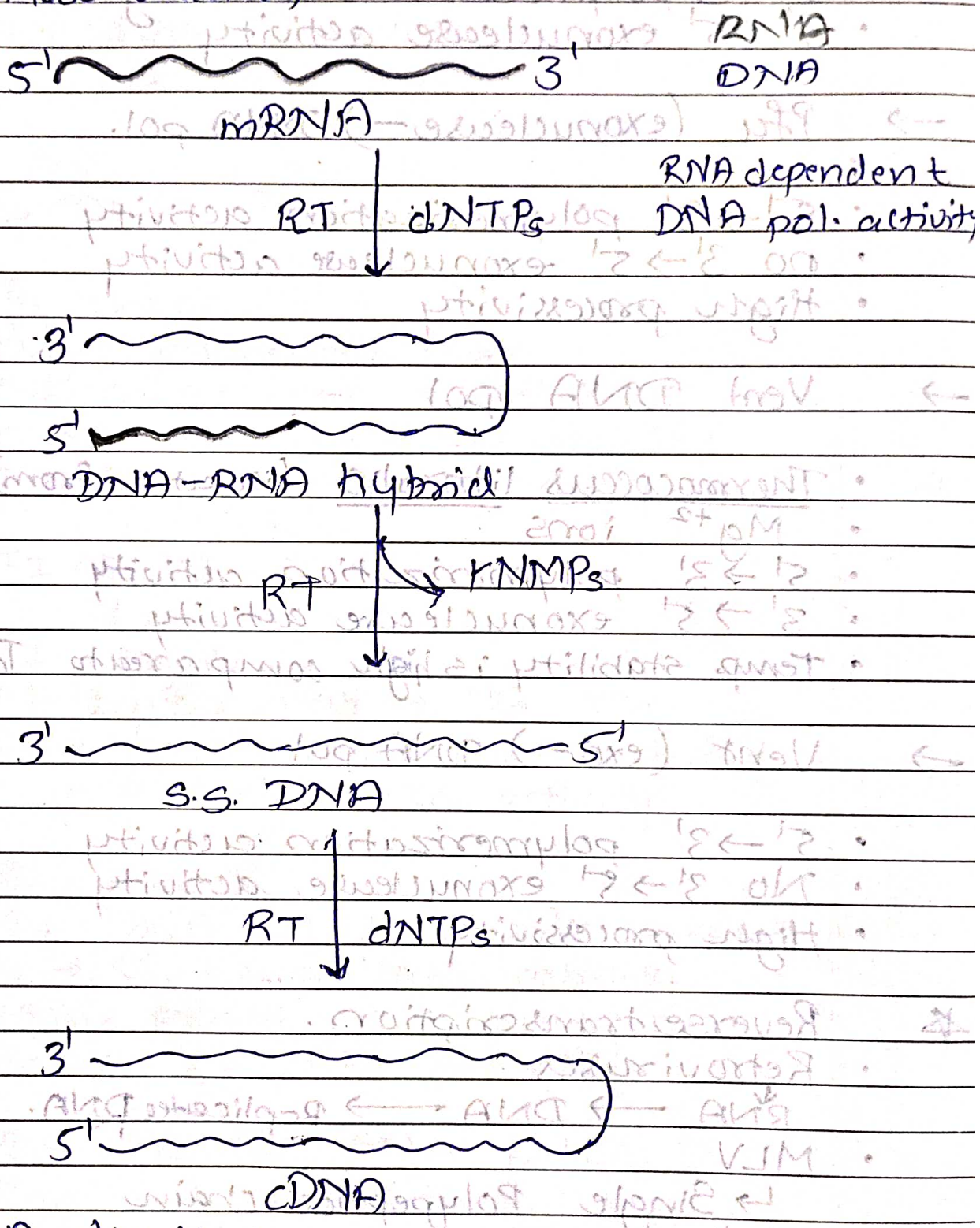
- 5'→3' polymerization activity
- No 3'→5' exonuclease activity
- High processivity

✱ Reverse transcription.

- Retroviruses
  RNA ⟶ DNA ⟶ Replicated RNA.
- MLV
  ↳ Single Polypeptide chain
- AMV
  ↳ Two polypeptide chain
- Cofactor for activation → Mg^{+2} or Mn^{+2} ions

- has RNA dependent DNA pol. activity
- has DNA dependent DNA pol. activity
- Also has RNase H activity

→ Mode of action



→ Applications

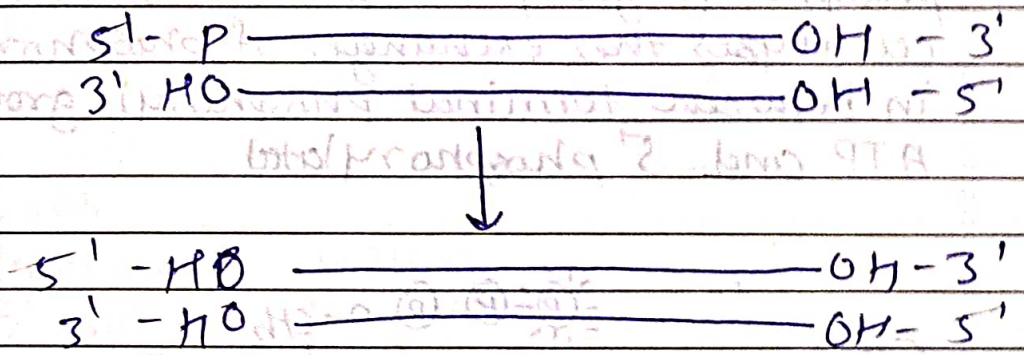
- cDNA library & RT. PCR
- In vitro synthesis of gene.

✡ Alkaline phosphatase

- Source ⇒ Bacteria or intestine of calf.
- Chemistry ⇒ dimeric glycoprotein, Zn^{+2}, alkaline pH
- Molecular mass ⇒ 14 kDa

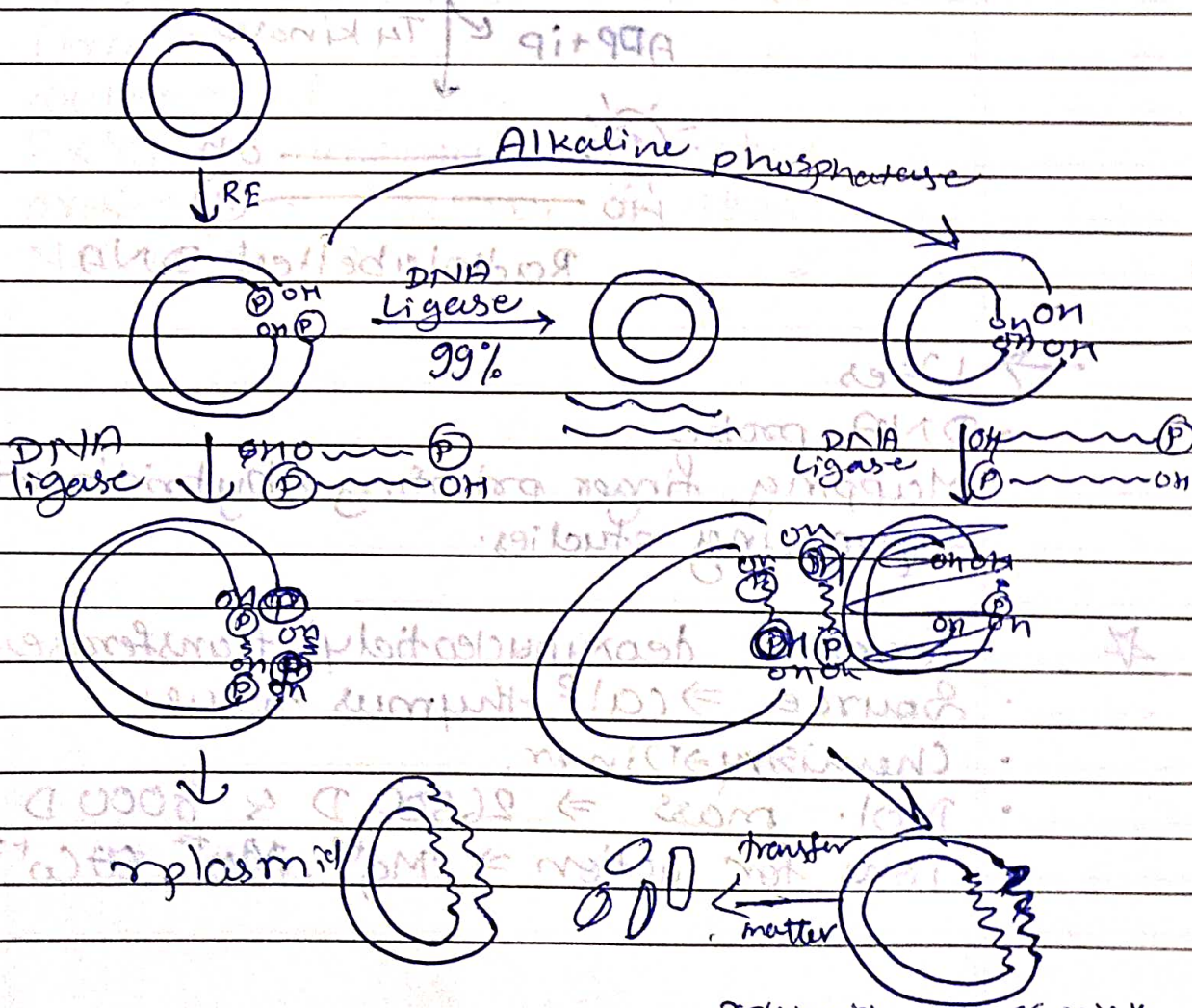
→ Mode of action.

- catalyzes removal of phosphoryl groups from 5' end of nucleic acid.



→ Uses

- prevents self-ligation of cloning vector.

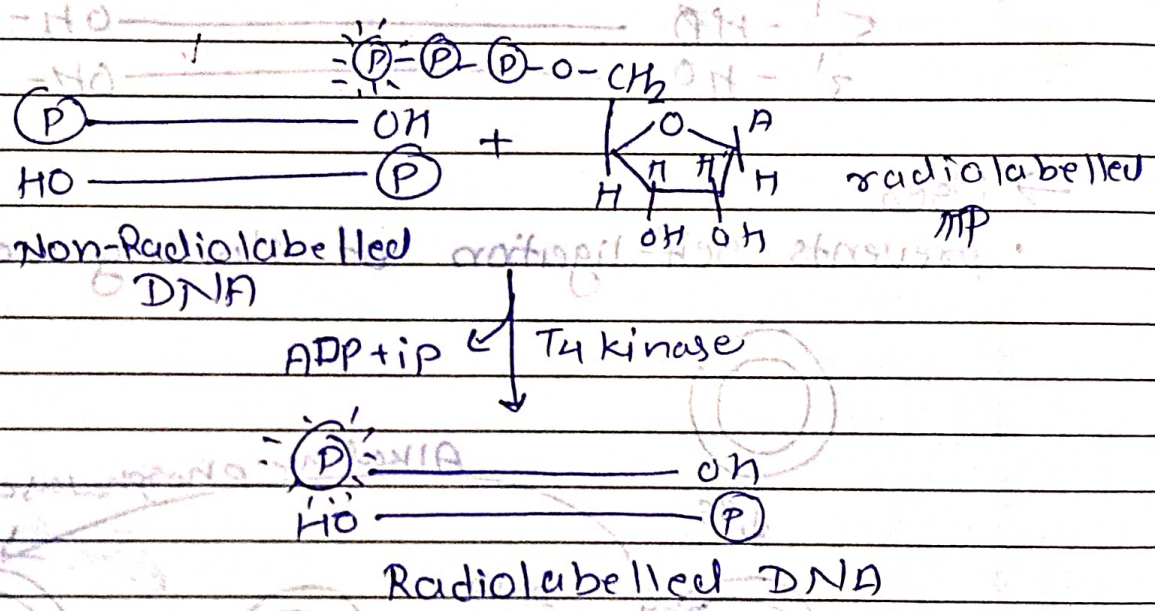


✡ T4 polynucleotidyl kinase

- Source ⇒ T4 phage
- Chemistry ⇒ Tetramer
- Mol. mass ⇒ 33 kDa.

→ Mode of action

- Catalyzes the phosphorylation of 5' end of nucleic acid by transferring terminal phosphoryl group of ATP to it. It even catalyses the exchange of phosphoryl group in between terminal phosphoryl group of ATP and 5' phosphorylated



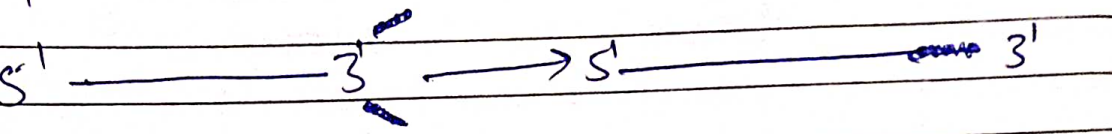
→ Uses

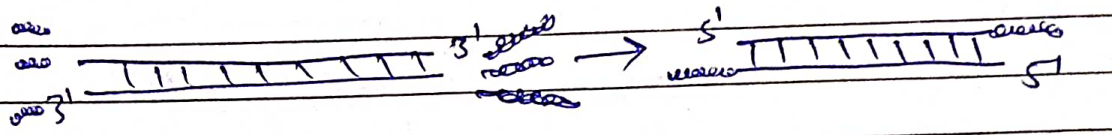
- DNA probe
- Mapping, finger printing, hybridization & sequencing studies.

✡ Terminal deoxynucleotidyl transferase.

- Source ⇒ calf thymus tissue
- Chemistry ⇒ Dimer.
- Mol. mass ⇒ 26500 D & 8000 D
- ions for action ⇒ Mg^{+2}, Mo^{+2}, Co^{+2}

→ Mode of action
- catcelyzes the addition of dNMPs from dNTPs to 3'-OH end of ss/ds DNA
- Often known as templete independent DNA polymerase

(i) 

(ii) 

→ Uses
- Homopolymer tailing.
- 3' end labelling
- In vitro mutagenesis

☆ Plasmid
- Double stranded
- Circular
- Supercoiled
- Extrachromosomal in nature
- present in bacteria & yeast.

→ Molecular size: